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Nitrogen Source Regulation of Growth and Photosynthesis in *Beta vulgaris* L.

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Sugar beets (*Beta vulgaris* L. cv F58-554H1) were grown hydroponically in a 16-h light, 8-h dark period at a photosynthetic photon flux density of 0.5 mmol m^{-2} s^{-1} for 4 weeks in half-Hoagland culture solution containing only nitrate-nitrogen. Half of the plants were then transferred to half-Hoagland solution with ammonium-nitrogen (7.5 mM), while the other half continued on 7.5 mM nitrate. Growth analysis was carried out by sampling the plants at 3-d intervals over a period of 21 d. Compared to plants supplied with nitrate, ammonium initially slowed the growth of shoots more than roots. Ammonium reduced both the area expansion of individual leaves and the relative water content of these leaves, but increased the amount of dry matter/area. The increase in specific leaf weight in ammonium-grown leaves was associated with a doubling of chloroplast volume, as much as a 62% rise in chlorophyll content, and a 4.3-fold higher accumulation of soluble protein. Ammonium nutrition substantially decreased the rate of expansion of photosynthetic (leaf) surface but did not decrease the rate of photosynthesis per area; in fact, net photosynthetic CO_2 exchange rates were slightly higher than in nitrate plants, due to the build-up in stromal enzymes of the Calvin cycle, several of which increased in total extractable activity on a leaf area basis, e.g. ribulose-1,5-biphosphate carboxylase oxygenase, sedoheptulose-1,7-biphosphatase. Nitrogen source had no effect on stomatal conductance. Rates of photosynthesis per chlorophyll were decreased slightly in ammonium-grown leaves, possibly due to an increased CO_2-diffusion resistance associated with the enlarged chloroplasts.

Non-legumes derive inorganic nitrogen from two principal sources, nitrate and ammonium. When plants are fed nitrate, they must first reduce the nitrate to ammonium via two energetically expensive steps of nitrogen assimilation, namely those associated with nitrate and nitrite reductases. Gas-exchange comparisons of nitrate- versus ammonium-supplied barley plants led Bloom et al. (1989) to suggest that nitrate assimilation commands up to 25% of either photosynthetic or mitochondrial electron transport capacity. In view of the increased requirements for photochemical energy for nitrate reduction, one might expect ammonium-grown plants to grow better than nitrate-grown plants under the same environmental conditions. However, the paradox is that ammonium-supplied plants generally exhibit poorer growth than nitrate-supplied plants, or plants supplied with a combination of nitrate and ammonium (Salsac et al., 1987) (there are some notable exceptions to this general rule, e.g. members of the acidophilic Ericaceae).

Why do plants grow less well under ammonium nutrition? The uptake of ammonium ions results in an efflux of hydrogen ions from roots (Marschner and Römheld, 1983), which increases the acidity of the culture medium around the roots. At low culture-solution pH (below 5.0) much nutrient uptake ceases, with the result that growth may suffer from nitrogen deprivation (Tolley-Henry and Raper, 1986). It is essential, therefore, to control culture-solution pH to determine how nitrogen source affects plant function independently of external pH effects (Rufty et al., 1983). Ammonium ions that enter the plant must be immediately assimilated to avoid toxic effects of ammonium on plant metabolism (ammonium is an in vitro uncoupler of photophosphorylation; Izawa and Good, 1972). The assimilation of ammonium proceeds via the GS-GOGAT pathway. In this pathway, GS produces substantial amounts of glutamine, which requires an equally large amount of α-ketoglutarate (from glycolysis and mitochondrial respiration of photosynthate) as the carbon skeleton necessary for glutamate formation (through GOGAT). This requirement for carbohydrate may influence ammonium toxicity by determining how fast the absorbed ammonium can be assimilated. Goyal et al. (1982) and Mehrer and Mohr (1989) observed that in seedling plants supplied with ammonium, the limited storage carbohydrate reserves quickly disappeared, necessitating the use of proteins and lipids as respiratory substrates; thus, they observed a rapid catabolism of chloroplast proteins and pigments. In pepper plants grown with low light, large amounts of unassimilated ammonium reached leaves, reducing photosynthetic rates and producing malformed thylakoids (Tàkacs and Técsi, 1992).

In ammonium-grown plants, PEP carboxylase activity in roots is increased compared to that in nitrate-grown plants (Arnozis et al., 1988; Cramer and Lewis, 1993). PEP carboxylase serves to generate hydrogen ions, which are necessary to replace those extruded from root cells in exchange for incoming ammonium ions (Arnozis and Findenegg, 1986). PEP carboxylase also serves an anapleurotic role by replacing oxaloacetate in the TCA cycle (oxaloacetate levels are depleted due to the removal of α-ketoglutarate in the assimilation of ammonium via the GS-GOGAT cycle). Because roots are dependent on shoots for carbohydrate and because ammonium assimilation results in a depletion of carbohydrate, it has been argued that root growth may be diminished

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Abbreviations: C_i, intercellular partial pressure of CO_2; DAT, days after initiating treatment; FBPase, fructose-1,6-bisphosphatase; GOGAT, glutamine:2-oxoglutarate aminotransferase; GS, glutamine synthase; PGA, 3-phosphoglycerate; SBPase, sedoheptulose-1,7-bisphosphatase; SLW, specific leaf weight; TCA, tricarboxylic acid cycle.

directly by loss of carbohydrate to ammonium assimilation within the root. Lindt and Feller (1987) proposed that reduced growth from culturing on ammonium arose from reallocation of phloem-derived carbon to nitrogen assimilation rather than root expansion growth. Lewis et al. (1989) also surmised that ammonium assimilation diverted carbon from root growth based on the reduced root elongation in wheat plants supplied NH_4^+ as opposed to NO_3^-.

Our objective in carrying out the present work was to investigate the mechanism by which ammonium diminished growth. Dry matter accumulation by plants is a function of the expansion of the photosynthetic surface and the intensity of photosynthesis per unit area of photosynthetic surface. The research approach we used compared the effects of ammonium-nitrogen supply with nitrate-nitrogen supply on plant growth, including the expansion of the leaf surface, and on photosynthetic gas exchange. Care was taken to minimize the effects of treatment on culture-solution pH by experimentally maintaining pH at 6.7 ± 0.2. We report that ammonium exerts its negative effects on growth through the rate of expansion of individual leaves and not through photosynthesis/area (although there was some reduction in the rate of photosynthesis/Chl).

MATERIALS AND METHODS

Plant Culture

Sugar beets (*Beta vulgaris* L. cv F58-554H1; U.S. Department of Agriculture, Salinas, CA) were sown in vermiculite and grown for 2 weeks with a 16-h light period at a PPFD of 0.5 mmol m^{-2} s^{-1}, at 25°C in growth chambers. The seedlings were transplanted to continuously aerated hydroponic culture with half-strength modified Hoagland solution (Terry, 1980) with NO_3^- as sole nitrogen source at 24 seedlings/15-L tank for an additional 2 weeks of pretreatment. After this month of growth, treatments were initiated: half the seedlings were transplanted at one plant per 20-L container (plastic lined) with 3.75 mM $Ca(NO_3)_2$ as sole nitrogen source, and the other seedlings were transplanted with 3.75 mM $(NH_4)_2SO_4$, all other nutrients as before except for 2.5 mM $CaCl_2$ added to the NH_4^+ mix. Solution pH was monitored at 2-d intervals and maintained at pH 6.7 ± 0.2 with additions of 1 M HCl or NaOH as needed. Nutrient solutions were replaced every 4 d and the ammonium-containing solutions were monitored for the presence of nitrate from microbial nitrification. No nitrate was detected in the nutrient solution or in the tissues of ammonium-grown plants. The plants were grown for 3 weeks after treatment was initiated and harvested every 3 d for growth analysis.

Growth Analysis

Plant parts were separated into leaf blades, petioles, fibrous roots, and storage roots, dried to constant weight at 60°C, and weighed. Individual area/leaf was measured immediately upon harvest with a Delta-T Devices (Santa Clara, CA) leaf area meter. Two fresh leaf discs (3.88 cm^2/disc) were subsampled from the most rapidly expanding leaf and dried for SLW determination at 60°C for 72 h. Initial relative growth rates ($d\ln Y/dt$ where Y = dry weight of shoot or roots) were obtained from linear regression through the first three time points of natural log-transformed growth data versus time, and expressed as g g^{-1} d^{-1}. Experimental results that were not log transformed were subjected to one-way analysis of variance to test for significant differences between treatments using Student's t test.

Cell Number and Cell Volume

The number of cells per unit leaf area and average leaf cell volume were determined by hemacytometer following extraction with 5% (w/v) chromic acid of rapidly expanding leaves from both nitrogen sources at the harvest 12 DAT as described by Terry (1980).

Chloroplast Numbers and Chloroplast Volume

Chloroplasts from leaves sampled at the harvest 12 DAT were isolated by the glutaraldehyde-NaCl method, counted on a hemacytometer, and measured with a micrometer according to Terry (1980).

Leaf Gas Exchange

At 12 DAT the rate of photosynthetic CO_2 uptake (P) per unit leaf area (P/area, μmol m^{-2} s^{-1}), stomatal conductance, and C_i (Pa) of individual, rapidly expanding leaves were determined over a range of ambient light intensities and CO_2 concentrations using open-flow gas exchange as described previously (Taylor and Terry, 1984). The P/C_i measurements were made by exposing the leaf initially to a saturating PPFD of 3 mmol m^{-2} s^{-1} and an ambient CO_2 concentration of 120 Pa for 1 h at 21 kPa O_2 and subsequently lowering the ambient CO_2 concentration to successive levels with 30 min at each ambient CO_2 level. Leaf temperature was maintained at 27.0 ± 0.5°C and monitored with a Yellow Springs Instrument (Yellow Springs, OH) model 43 Telethermometer and model 421 thermocouple attached to the underside of the leaf. RH was maintained at 65%. PPFD was held at either a constant level or increased gradually to achieve light saturation at each CO_2 level and measured with a Li-Cor 185-S quantum radiometer.

Calvin Cycle Enzymes

Two leaf discs (3.88 cm^2/disc) from each of four replicate plants of each of the two nitrogen treatments were harvested into liquid nitrogen and extracted as described previously (Rao and Terry, 1989), except that centrifuged extracts were desalted in a cold room (4°C) on Sephadex G-25M columns (Pharmacia) prior to analysis. Initial (assayed immediately after desalting) and total (assayed after 10 min of incubation with saturating CO_2 and Mg^{2+}) activities of Rubisco were assayed by the coupled-enzyme assay as modified by Sharkey et al. (1991). For all other enzymes of the Calvin cycle, total activities were obtained by including 50 mM DTT in the extraction medium. Initial activity was determined in the absence of DTT both in the extraction and assay medium as described previously (Rao and Terry, 1989).

Leaf Chl and Soluble Protein

Chl was measured in 80% (v/v) acetone extracts (Arnon, 1949) from the rapidly expanding leaf used for SLW and cell-property determinations so as to facilitate direct comparisons. Foliar soluble protein was estimated by the dye-binding method of Bradford (1976) using BSA as a standard.

Chemicals

All compounds used were purchased from Sigma with the exception of CO_2 and O_2 standard gases (Matheson Gas Products, Secaucus, NJ).

RESULTS

When nitrogen was supplied as ammonium, the growth of roots (fibrous roots and storage root) and the growth of shoots (leaf blades and petioles) were slower than when nitrogen was supplied as nitrate (Fig. 1). By the final harvest, 21 DAT, the total dry weight of nitrate plants was 2.1 times larger than that of ammonium-treated plants. With respect to shoot dry weight, the initial relative growth rate was 0.331 g g^{-1} d^{-1} for nitrate-supplied plants and 0.238 g g^{-1} d^{-1} for ammonium-supplied plants. Initial root growth was less affected by ammonium treatment than initial shoot growth: the initial relative growth rate of nitrate-supplied roots was 0.393 g g^{-1} d^{-1} compared to ammonium roots' relative growth rate of 0.348 g g^{-1} d^{-1}. Over the first 15 d the root:shoot ratio tended to be higher in the ammonium-treated plants, but by 21 d the root:shoot ratio was higher in nitrate plants, primarily because of the rapid development of the storage root (Table I). The proportion of fibrous root dry weight of the total root dry weight decreased from about 55% at d 3 to about 26% at d 21, but was little affected by nitrogen source (Table I).

With ammonium as nitrogen source, both the total leaf dry matter/plant and leaf area/plant were less relative to nitrate-grown plants (Fig. 2, a and b, respectively). Since the number of leaves per plant did not differ between the two treatments (data not shown), the effect of ammonium supply was on the rate of expansion of individual leaves, which was increased by nitrate versus ammonium supply, rather than on the rate of leaf production. The ratio of petiole to lamina weight was unchanged with time in ammonium-grown plants and increased with time in nitrate-supplied plants (Fig. 2d). This was because nitrate-grown leaves not only expanded their leaf blades faster, but also elongated their petioles more than ammonium-grown leaves (as was evident from visual examination of the plants). Ammonium-grown leaves had significantly higher SLW than nitrate-supplied leaves (Fig. 2c). Over the 3-week harvest period (0–21 DAT), SLW increased with time in both treatments; nitrate leaves' SLW rose from 26 to 41 g m^{-2}, whereas in ammonium leaves SLW increased from 26 to 53 g m^{-2}.

Total Chl per area was consistently higher in ammonium leaves compared to nitrate leaves (Fig. 3). Chl contents in ammonium-treated plants increased over the first 15 d from 0.31 to 0.57 g m^{-2}, and then decreased slightly. In nitrate plants, however, there was no change with time, with Chl averaging 0.35 g m^{-2}. Chl contents expressed per unit leaf dry weight were also higher in ammonium-grown than in

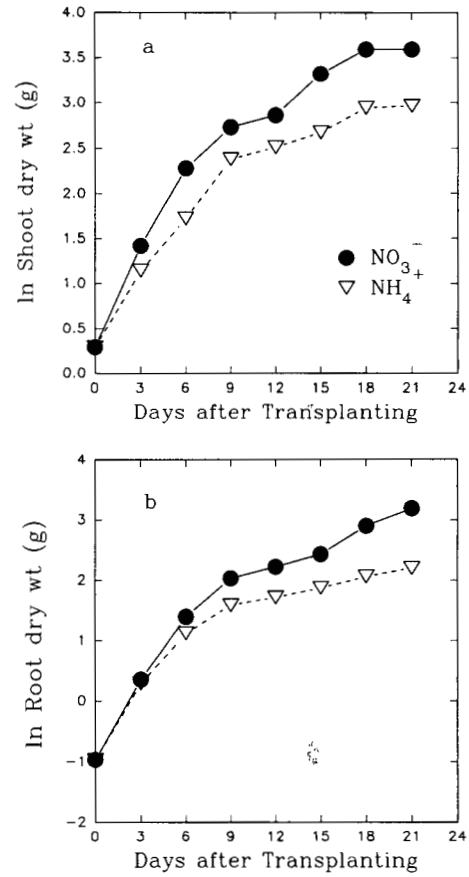

Figure 1. Natural-log-transformed growth curves for the shoot dry matter accumulation (leaf blades + petioles) (a) and root dry matter accumulation (fibrous + storage root) (b) of sugar beets supplied with either nitrate or ammonium. Values are means of three replicate plants for each treatment.

nitrate-grown leaves. There was no significant effect of nitrogen source on the Chl *a/b* ratio, although the values in ammonium leaves were slightly higher (data not shown). Over time, the Chl *a/b* ratio dropped in both treatments from 4.0 to 3.4.

The effect of nitrogen source on cell and chloroplast attributes was determined by measuring numbers and volumes of cells and chloroplasts from rapidly expanding leaves of each treatment. These leaves were about 1.5 to 1.75 dm^2 in area and were similar to those used in the photosynthesis measurements (below). The plants were equivalent in size to the plants at 12 DAT. When leaves from plants of the two treatments were compared in this way, i.e. at the same physiological stage of growth, they contained similar numbers of cells per unit leaf area and comparable average cell volumes; furthermore, the number of chloroplasts per unit leaf area was unaffected by nitrogen source (Table II). However, the average volume of chloroplasts was increased substantially (significant at $P < 0.01$) by ammonium treatment, i.e. 43 μm^3 versus 22 μm^3 for nitrate-supplied plants. When sampled on the same harvest at 12 DAT, foliar soluble protein was 4-fold higher on a leaf area basis in ammonium-grown

Table I. *Dry weights of storage root and fibrous roots and total root:shoot ratio of sugar beets cultured on nitrate or ammonium*

Values are mean ± SD, $n = 3$. Fibrous root dry weight also expressed as percentage of total root dry weight.

Time	Treatment	Storage Root Dry Weight	Fibrous Roots Dry Weight	Root/Shoot
DAT		*g*	*g*	*g g*$^{-1}$
3	NIT[a]	0.63 ± 0.23	0.79 ± 0.40 (56%)	0.34
	AMM[b]	0.60 ± 0.12	0.73 ± 0.19 (55%)	0.42
6	NIT	2.33 ± 0.70	1.68 ± 0.57 (42%)	0.41
	AMM	1.78 ± 0.32	1.30 ± 0.24 (42%)	0.55
9	NIT	4.64 ± 1.17	2.95 ± 0.46 (39%)	0.50
	AMM	2.83 ± 0.45	2.02 ± 0.25 (42%)	0.45
12	NIT	5.34 ± 0.84	3.87 ± 0.51 (42%)	0.39
	AMM	2.91 ± 0.30	2.62 ± 0.42 (47%)	0.54
15	NIT	7.46 ± 0.86	3.95 ± 0.20 (35%)	0.41
	AMM	3.60 ± 1.22	2.88 ± 0.94 (44%)	0.45
18	NIT	12.3 ± 3.68	5.82 ± 1.20 (32%)	0.50
	AMM	5.17 ± 1.48	2.66 ± 0.25 (34%)	0.41
21	NIT	18.1 ± 4.41	6.08 ± 1.13 (25%)	0.67
	AMM	6.59 ± 3.24	2.47 ± 0.30 (27%)	0.47

[a] NIT, Nitrate. [b] AMM, Ammonium.

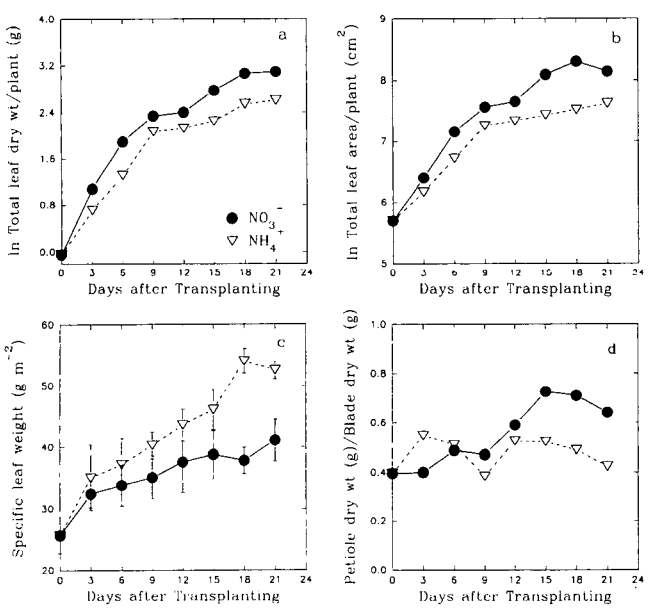

Figure 2. Natural-log-transformed growth curves for total leaf blade dry-matter accumulation (a) and total leaf area per plant of sugar beets (b) supplied with either nitrate or ammonium. (Values are means of three replicate plants for each treatment.) c, Time course for the specific leaf weight (g dry matter m^{-2} leaf area) of rapidly expanding leaves of sugar beet plants supplied with either nitrate or ammonium. Values are mean ± SD, $n = 4$ determinations. d, Petiolar dry matter accumulation as a proportion of leaf blade dry matter (g/g) from sugar beet plants supplied with either nitrate or ammonium. Values are means of three replicate plants for each treatment.

plants (29.8 ± 5.57 g m^{-2}) than nitrate-supplied sugar beets (6.94 ± 0.868 g m^{-2}).

At ambient CO_2 levels (32 Pa), the net rate of photosynthesis per area at most irradiances was slightly higher in ammonium-grown than in nitrate-grown plants (Fig. 4a). However, on a per Chl basis, rates of photosynthesis were less in ammonium-grown than in nitrate-grown sugar beets (Fig. 4b). Total foliar Chl for the two treatments at the time of the gas-exchange measurements was 0.545 ± 0.039 and 0.353 ± 0.034 g Chl m^{-2} for ammonium and nitrate plants, respectively (Fig. 3). Nitrogen source had no effect on stomatal conductance of intact sugar beet leaves (Fig. 4c), whereas intercellular CO_2 concentration was lowered in ammonium-grown compared to nitrate-grown leaves (Fig. 4d). Dark respiration was not significantly different in nitrate-cultured sugar beets compared to ammonium cultured plants (data not shown).

At high ambient levels of CO_2 (100 Pa) and photon fluxes above 1 mmol m^{-2} s^{-1}, the net rate of photosynthesis per area was substantially higher in ammonium-grown than in nitrate-grown plants (Fig. 5a), but the ammonium leaves were still slightly lower on a per Chl basis (data not shown). The relationship of photosynthesis with CO_2 level was explored further by considering the net rate of photosynthesis at light saturation as a function of intercellular CO_2 pressure (Fig. 5b). The results showed that nitrate-grown leaves reached CO_2 saturation at about 75 Pa, whereas ammonium-grown leaves did not reach CO_2 saturation even at 105 Pa. Thus, ammonium-grown leaves appeared to require higher levels of CO_2 to achieve maximum photosynthesis.

Six photosynthetic carbon reduction cycle enzymes were assayed in ammonium-grown and nitrate-grown leaves (Table III). Of these six enzymes, Rubisco and SBPase were found exclusively in the chloroplast; FBPase activity (due to

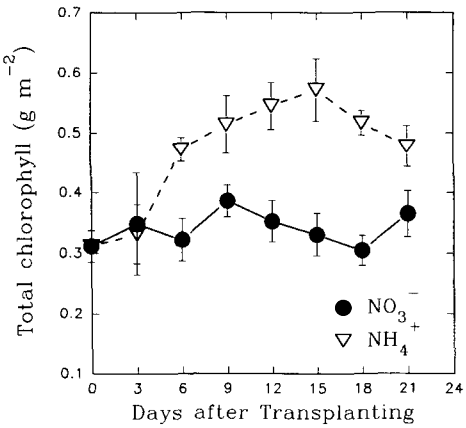

Figure 3. Total Chl per unit leaf area (g m^{-2}) of rapidly expanding leaves from sugar beet plants grown with either nitrate or ammonium. Values are mean ± SD, n = 4 determinations.

the assay conditions) is assumed to be mostly chloroplastic, whereas the activity of each of the remaining three enzymes may include a substantial cytosolic component in addition to the chloroplastic component. When the activities of these enzymes were compared on a Chl basis, the results showed no significant differences with regard to nitrogen source. When the activities were compared on a leaf area basis, the results suggested that the total activities of each of these enzymes was increased in most instances by about 35 to 40% as a result of supplying ammonium instead of nitrate (Table III), with Rubisco and SBPase demonstrating significant increases ($P < 0.05$ and $P < 0.01$, respectively). FBP aldolase activity was increased ($P < 0.05$) most by ammonium, with its total activity increasing more than 2-fold. The initial or in vivo activities of these enzymes were similarly affected by ammonium, except for PGA-kinase (Table III).

DISCUSSION

Growth Reduction with Ammonium Nitrogen Supply

Why do sugar beet plants supplied with ammonium rather than with nitrate suffer reduced rates of growth? Our results

Table II. *Effects of nitrogen source on some sugar beet leaf and chloroplast attributes from plants grown 12 d after transplanting to either nitrate or ammonium*

Values are mean ± SD, n = 12 for nitrate, n = 18 ammonium, except for soluble protein, where n = 4 for both treatments.

	Nitrate	Ammonium
No. of cells per unit leaf area (10^{10} m^{-2})	1.23 ± 0.36	1.10 ± 0.15
Mean leaf cell volume (10^{-14} m^3)	2.66 ± 0.94	2.80 ± 0.46
No. of chloroplasts per unit leaf area (10^{12} m^{-2})	0.98 ± 0.21	0.87 ± 0.29
Chloroplast volume (μm^3)	22.3 ± 3.53	43.0 ± 10.0[a]
Foliar soluble protein (g m^{-2})	6.94 ± 0.868	29.8 ± 5.57[a]

[a] Means significantly different ($P < 0.01$).

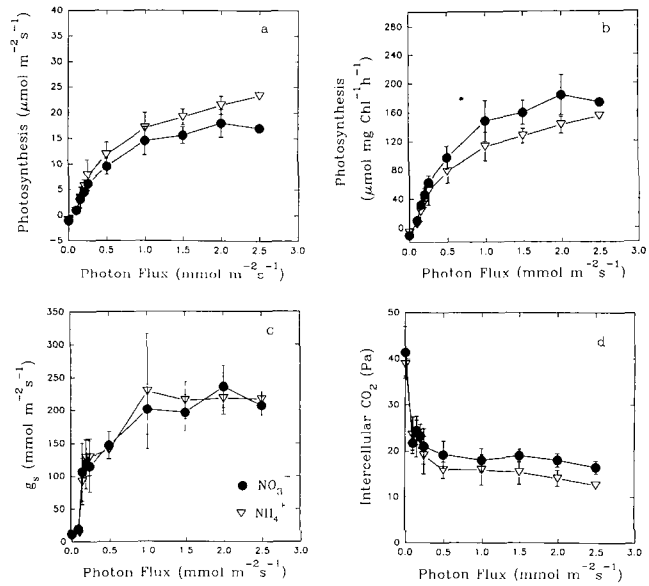

Figure 4. Influence of nitrogen source on the relationship with photon flux density for the rate of photosynthesis per unit area (a), rate of photosynthesis per Chl (b), stomatal conductance (c), and intercellular CO_2 partial pressure (d). Photosynthesis was measured as leaf CO_2 uptake at 27 ± 0.5°C, air levels of CO_2 (32 Pa), and O_2 (21 kPa). Values are mean ± SD, n = 4 determinations.

show that the immediate effect of supplying ammonium was to preferentially decrease shoot growth compared to root growth. van Beusichem et al. (1988) also observed a prejudicial effect of ammonium (versus nitrate) nutrition on shoots relative to roots in *Ricinus communis*. Other researchers have found ammonium nutrition to decrease root dry matter accumulation preferentially, as in barley (Lewis and Chadwick, 1983), cucumber (Lindt and Feller, 1987), the CAM plant *Kalanchoë blossfeldiana* (Ota et al., 1988), and wheat (Lewis et al., 1989). Ammonium nutrition decreased shoot growth in sugar beets through decreased growth of individual leaves rather than through decreased leaf production by the terminal bud. This was also observed for the nitrophilic weed *Urtica dioica* (Rosnitschek-Schimmel, 1982). Ammonium supply had marked negative effects on individual leaf expansion in *Kalanchoë* (Ota et al., 1988) and in *Moricandia arvensis* (Winter et al., 1982). Nitrogen source had no effect on the number of cells per area or average leaf cell volume (Table II). However, leaves of the two treatments were compared at a similar stage of development (i.e. those leaves 1.5 to 1.75 dm^2 in area that were undergoing maximum rates of expansion) when cells were comparable in size. Most of the expansion of individual leaves is due to cell expansion growth (Terry, 1970), and leaves almost certainly grew more slowly under ammonium nutrition because cells expanded more slowly.

It is not clear why ammonium caused such a marked reduction in leaf cell expansion. Ammonium may exert its primary influence on leaf expansion through an effect on epidermal turgor (Shackel et al., 1987; Waldron and Terry, 1987). Osmolytes contributing to leaf expansion include NO_3^-, Glc, Suc, and organic acids (Blom-Zandstra and

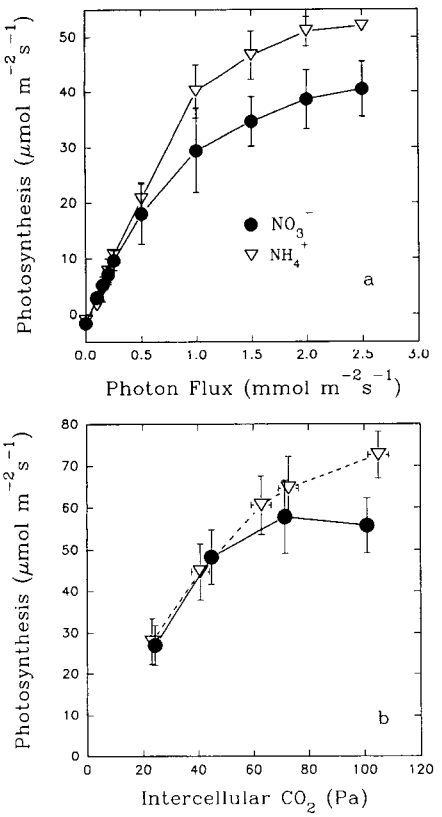

Figure 5. a, Photosynthesis as a function of photon flux density for sugar beet plants grown with either nitrate or ammonium. Plants were measured at an external CO_2 level of 100 Pa. Values are mean ± SD, n = 4 determinations. b, Photosynthesis as a function of internal CO_2 partial pressure in sugar beet plants grown with either nitrate or ammonium. Measurements were obtained at a saturating photon flux of 3 mmol m^{-2} s^{-1}. Values are mean ± SD, n = 6 determinations.

Lampe, 1985; Steingrover et al., 1986). In the former study, Blom-Zandstra and Lampe (1985) grew lettuce plants at various light intensities and found that as concentrations of sugars and organic acids dropped with decrease in light intensity, NO_3^- accumulated in leaves to maintain constant osmolarity. In spinach, Steingrover et al. (1986) found that leaf elongation rates depend on the capacity for nitrate uptake and subsequent accumulation in the leaves. Under ammonium nutrition, sugar beet leaves accumulate no nitrate and much less sugars and cations (e.g. magnesium, potassium ions) (T.K. Raab and N. Terry, unpublished data), so that there was most likely a shortage of osmolytes for cell expansion. With ammonium nutrition, leaves had somewhat smaller water contents than nitrate-grown leaves (about 81% for ammonium-grown versus 87% for nitrate-grown leaves).

What is the cellular basis for the steady build-up in SLW in ammonium plants (Fig. 3c)? Most of the accumulation in SLW in ammonium-grown leaves is attributable to increased protein. Soluble leaf protein increased 4.3-fold in ammonium-grown compared to nitrate-grown leaves. Evans (1989) estimated that 75% of leaf total nitrogen is contained within chloroplastidic protein. Thus, a marked increase in foliar protein would most likely be distributed among stromal enzymes and light-harvesting Chl protein complexes of PSI and PSII. Our results with sugar beet show that the build-up in soluble leaf protein with ammonium nutrition was associated with up to a 62% increase in Chl (which is associated with thylakoid proteins), a 40% build-up in some photosynthetic stromal proteins (e.g. Rubisco), and a doubling in chloroplast volume. Golvano et al. (1982) also observed ammonium to increase both the size and soluble protein content of wheat chloroplasts. Thus, the increase in SLW with ammonium nutrition appears to be associated with substantial protein accumulation and with chloroplast enlargement.

Gas-Exchange Characteristics with Ammonium Nitrogen Supply

Given that ammonium is an uncoupler of photophosphorylation in chloroplasts (Izawa and Good, 1972), diminished rates of photosynthesis/area (P/area) could occur if ammonium ions were transported to leaves in sufficient quantity. However, P/area was increased slightly rather than decreased by ammonium nutrition. The increase in P/area, especially at high ambient CO_2, was the result of increased amounts of photosynthetic apparatus per unit leaf area: chloroplasts were larger and Chl and some photosynthetic enzymes, as well as soluble leaf protein, were in greater amounts in ammonium-grown leaves. Ammonium had no effect on stomatal conductance but did reduce intercellular CO_2 concentration (through increased CO_2 utilization in photosynthesis).

Table III. *Activities of Calvin cycle enzymes from leaves of sugar beet plants grown 12 d after transplanting to either nitrate or ammonium as nitrogen source*

Values are mean ± SD, n = 4 determinations. For Rubisco, initial activities are from desalted extracts assayed immediately, whereas total activities represent extracts incubated for 10 min with saturating CO_2 and Mg^{2+} prior to assay. For PGA-kinase, FBPase, and SBPase, initial activities were obtained without DTT in either extraction or assay medium, whereas total activities were assayed with 50 mM DTT.

	Nitrate	Ammonium
	μmol m^{-2} leaf area s^{-1}	
Rubisco		
Initial	132 ± 24.3	150 ± 47.0
Total	171 ± 34.6[a]	237 ± 20.5[a]
PGA-kinase		
Initial	195 ± 16.0	180 ± 25.5
Total	364 ± 70.3	500 ± 74.3
FBPase		
Initial	2.92 ± 0.79	4.52 ± 1.77
Total	12.4 ± 1.98	20.3 ± 6.53
SBPase		
Initial	5.23 ± 1.58	7.83 ± 2.45
Total	9.89 ± 0.16[b]	14.2 ± 1.39[b]
FBP aldolase	22.1 ± 8.33[a]	48.8 ± 6.44[a]
Transketolase	22.7 ± 4.30	30.4 ± 3.30

[a, b] Means significantly different by t test at $P < 0.05$ and $P < 0.01$ levels, respectively.

At high intercellular levels of CO_2 (Pa) and at irradiances approaching saturation, P/area was up to 31% higher in ammonium-grown than in nitrate-grown plants and did not reach CO_2 saturation even at 105 Pa (Fig. 5b). The most likely explanation for the increase in P/area at high CO_2 is that levels of photosynthetic enzymes were higher so that more CO_2 was required to saturate the photosynthetic apparatus: the total activity of Rubisco increased by 38%, of FBPase by 65%, and of SBPase by 44% in ammonium versus nitrate leaves, whereas Chl content increased up to 62%. Thus, at low CO_2 photosynthesis is CO_2 limited and differs little between treatments (Fig. 5b), whereas at high CO_2 photosynthesis is enzyme limited and is higher in ammonium leaves, which have more photosynthetic apparatus per leaf area.

On a per Chl basis, rates of photosynthesis were lower in ammonium-grown than in nitrate-grown leaves. When the data of Figure 5b are plotted as photosynthesis/Chl (P/Chl) versus C_i, P/Chl is much lower in ammonium than in nitrate leaves at low C_i and increases more slowly with increases in C_i (data not shown). At high C_i, P/Chl values for the two treatments are not significantly different from one another. One explanation for these results is that there is an increased diffusion resistance for CO_2 in ammonium leaves, which is associated with the enlarged chloroplasts. The doubling of chloroplast volume in ammonium leaves may have increased the distance and tortuosity of CO_2 molecules before they reach the active sites of Rubisco.

From CO_2-response curves of spinach plants grown at different nitrate-supply levels, Evans and Terashima (1988) derived a CO_2-transfer resistance (i.e. from the intercellular air spaces to the site of carboxylation) of 2.2 m^2 s bar mol^{-1} CO_2. Such a resistance would simply lower in vivo Rubisco activities, since leaves of higher nitrogen status would have higher amounts of enzyme per unit leaf area and lower active-site CO_2 partial pressure. Furthermore, soluble leaf protein levels are 4.3-fold larger in ammonium leaves, whereas total Rubisco activities are only 38% higher. Therefore, the chloroplasts of ammonium leaves may contain Rubisco in inactive form or as unassembled Rubisco subunits, another factor likely to increase the tortuosity of CO_2 molecules diffusing through the chloroplasts. At very high CO_2 levels, the CO_2 limitation associated with the enlarged chloroplasts is apparently overcome and P/Chl values of ammonium leaves approach those of nitrate leaves.

Eliminating the two energetically expensive steps associated with nitrate and nitrite reductases by supplying ammonium did not result in increased growth. Even though nitrate assimilation may require up to 25% of either photosynthetic or mitochondrial electron transport (Bloom et al., 1989), there was no evidence from our results (i.e. P/Chl values were higher in nitrate leaves) that the consumption of photochemical energy for nitrate reduction limited photosynthetic CO_2 fixation. We conclude that the effect of ammonium nutrition in diminishing growth is related not to an effect on photosynthesis but to an effect on leaf expansion, the exact mechanism for which remains to be determined. The results also suggest that chloroplasts act as a major storage repository for excess nitrogen; under ammonium nutrition, chloroplasts double in volume and accumulate huge amounts of protein from amino acids imported from the roots via the xylem.

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